A unique short signal sequence in membrane-anchored proteins of Archaea

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A unique short signal sequence in membrane-anchored proteins of Archaea

Sir,

Detailed information is available on the signal sequences of bacteria and eukaryotes that direct secretory proteins out of the cell (von Heijne, 1990, *J Membr Biol* **115:** 195–201). It is generally assumed that such signals in Archaea are identical to the bacterial and eukaryotic signals. Recently, we have identified a glucose-binding protein in *Sulfolobus solfataricus* that functions as a subunit of an ABC type of sugar transporter (Albers *et al*., 1998, submitted). The binding protein is secreted by the cells in order to interact with the extracellular sugars. Unlike binding proteins in Gram-negative bacteria, the protein remains anchored to the membrane by means of an amino-terminal transmembrane segment. In this respect, it also differs from binding proteins of Gram-positive bacteria that use cysteine-linked lipid anchors for membrane binding. The determined N-terminal amino-acid sequence of the purified glucose binding protein completely matched the sequence predicted from the DNA sequence from the database of *S. solfataricus*, except that the first 12 predicted amino acids were lacking in the purified protein. The protein appears to be processed at a glycyl–leucyl peptide bond that precedes a putative transmembrane segment thought to anchor the secreted protein to the membrane. Although the program SIGNALP (Nielsen *et al*., 1997, *Protein Eng* **10:** 1–6; http://www.cbs.dtu.dk/services/SignalP/) predicts an eukaryotic-type signal peptide cleavage site at amino acids 36–37 (Gly–Phe), a prokaryotic-type (Gram positive) signal peptide cleavage site at amino acids 43–44 (Pro–Ser), and a prokaryotic-type (Gram negative) one at amino acids 47–48 (Ala–Val), none of these sites are used. Cleavage at this position resembles strikingly that of the flagellins of methanogenic and halophilic Archaea (Kalmokoff and Jarrell, 1991, *J Bacteriol* **173:** 7113–7125; Faguy *et al*., 1996, *J Bacteriol* **178**: 902–905). These secreted proteins bear a very short hydrophilic, positively charged N-terminal sequence of 11 or 12 amino acids (Fig. 1) that is processed at a glycine followed by a stretch of hydrophobic amino acids that seems to be involved in the intramolecular flagellin interactions.

The existence of such a short signal peptide in a secretory protein is exceptional. Therefore, we have searched the genomic databank of Archaea to determine whether the Gly–Leu motif is more commonly found in secretory proteins. From these searches, it appears that secretory proteins can be classified in two main classes, i.e. one class that exhibits a typical bacterial signal sequence and one that shows a glycine followed by a long hydrophobic stretch of amino acids. Only the latter class of proteins was further analysed. Main examples are the cutinases and amylases from *Pyrococcus horikoshii* OT3 and *S. solfataricus* P2, and the maltose/trehalose-binding

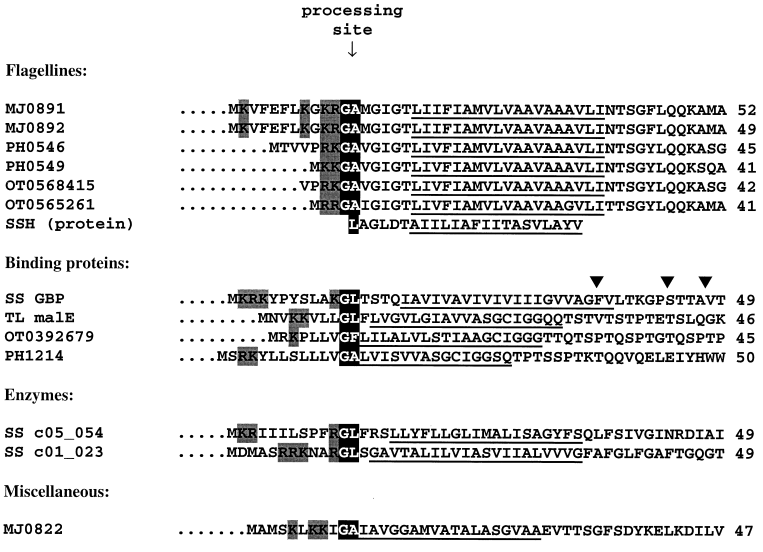

Fig. 1. List of archaeal genes containing the unique procession site. The positively-charged amino acidsare shaded grey and precede the cleavage sites, which are shadowed black. Stretches of hydrophobic amino acids are underlined. Accession numbers are designated MJ for *Methanococcus janashii*, PH for *Pyrococcus horikoshii* OT3, SS for *Sulfolobus solfataricus*, SSH for *Sulfolobus shibatae*, OT for *Pyrococcus* OT3 and TL for *Thermococcus litoralis*. MJ0822, S-layer protein; PH1214, sugar-binding protein; TL malE, maltose-binding protein; SS GBP, glucose-binding protein; OT0392679, multiple sugar-binding protein; SS c05_054 cutinase; SS c01_023, serine cutinase. The ▼ indicates putative signal peptidase cleavage sites in the glucose-binding protein of *S. solfataricus*. See text for further details.

protein from *Thermococcus litoralis* (Xavier *et al*., 1996, *J Bacteriol* **178:** 4773–4777). It should be emphasized that only for flagellins and for the glucose-binding protein has the exact location of the cleavage site been determined biochemically. In none of the other cases is there compelling evidence available for processing at the indicated site. However, these proteins have in common that the positively charged amino terminus connects to a stretch of hydrophobic amino acids through a glycine (Fig. 1). Taken together, these data suggest that some secreted proteins in *Sulfolobus solfataricus* and other archaea are transported by a similar mechanism to archaeal flagellins and not by the sec-dependent pathway.

Kalmakoff and Jarrel (1991, *J Bacteriol* **173:** 7113–7125) have shown that archaeal flagellins are not homologous to bacterial flagellins, but rather resemble the bacterial proteins of the type IV pilin superfamily, which also includes DNA uptake and protein excretion systems (Hobbs and Mattick, 1993, *Mol Microbiol* **10**: 233–243). Proteins that are secreted via these systems show a high homology at their N-termini with a well-conserved glycine at position −1 and a glutamate at the +5 position (Bairoch, 1991, *Nucleic Acids Res* **20:** 2013–2018). In the case of the archaeal systems, this glutamate residue is not conserved and only found in the flagellum of *Methanospirillum hungatei* (Faguy *et al*., 1994, *J Bacteriol* **176:** 7491–7498). The genome of *S. solfataricus* bears a homologue of VirB, a component of a DNA uptake system in *Agrobacterium*. The open reading frame located downstream of *Methanococcus voltae* flagellins and the gene MJ0900 of *Methanococcus janaschii* show significant homologies to pilB (Bayley and Jarrell, 1998, *J Mol Evolution* **46:** 370–373), a nucleotide-binding protein involved in pilus function in *Pseudomonas aeruginosa* (Hobbs and Mattick, 1993, *Mol Microbiol* **10:** 233–243). Furthermore, there are two more pilB homologues (MJ0781 and MJ1287) found in *M. janaschii*. This demonstrates that type IV secretion systems are also present in Archaea.

The current model for the secretion of archaeal flagellins across the cytoplasmic membrane (Jarrell *et al*., 1996, *J Bacteriol* **178**: 5057–5064) resembles the proposal for type IV pili (Mattick and Alm, 1995, *Trends Microbiol Sci* **3**: 411–413). The flagellins are guided to the membrane by chaperones that prevent non-specific interactions of the flagellins at their hydrophobic N-termini. At the membrane, the charged part of the N-terminus is cleaved off by a cytosolic peptidase. In the case of type IV pilin proteins, the prepilin peptidase pilD removes the signal peptide and directly N-methylates the leucine of the pilins (Nunn and Lory, 1991, **88**: 3281–3285; Strom *et al*., 1993, *Proc Natl Acad Sci USA* **90**: 2404–2408). Archaeal flagellins appear not to be methylated, but after translocation across the membrane the proteins are glycosylated and finally assemble into a flagellum. Whether there is a stage in which the flagellins are free in the periplasm or whether they reach the flagellum by lateral diffusion in the membrane is not known. The positively charged amino terminus of the precursor form probably blocks membrane translocation, and it may well be that processing is needed to allow translocation of the flagellin across the membrane.

The above model for the transport of flagellins explains the orientation of the membrane-anchored glucose-binding protein in *S. solfataricus*. After translocation of its catalytic binding domain across the membrane, the charged amino terminus may be cleaved by the same peptidase that is involved in processing of flagellins. Moreover, the glucose-binding protein is glycosylated like the flagellins. It is not yet clear whether the glucose-binding protein remains membrane anchored or assembles into a large complex in the 'periplasm' of *S. solfataricus*. However, its release from the membrane requires substantial amounts of detergent, which indicates that it is membrane integrated.

Taken together, these data suggest that membrane-anchored secretory proteins in Archaea are processed and translocated by a mechanism that resembles that of archaeal flagellins.

Note added in proof

Pedgen *et al.* (1998, *J Bacteriol* **180:** 5921–5927) have recently described a cellulose-binding protein of the Gram-positive bacterium *Ruminococcus albus* which also belongs to the type IV pilin protein family. The signal sequence is eight amino acids long, highly positively charged, and cleaved at a glycine followed by a phenylalanine.

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The *Escherichia coli* haemolysin secretion apparatus: a potential universal antigen delivery system in Gram-negative bacterial vaccine carriers

Sir,

The *Escherichia coli* α-haemolysin (HlyA) secretion apparatus is the prototype of a type I secretion system